

We claim:

1. A process for directionally ligating a double-stranded nucleic acid to a first adaptor sequence, the process comprising:
  - a. forming an amplification product from the double-stranded nucleic acid using a mixture comprising (i) a polymerase, (ii) a deoxynucleotidetriphosphate (dNTP) mixture, the dNTP mixture comprising modified dNTPs for at least one of the four nucleotide triphosphates comprising dATP, dGTP, dCTP, dTTP and analogs thereof which, when incorporated into a polynucleotide, impart resistance against enzymatic degradation by an exonuclease at the site of incorporation of the modified dNTPs, (iii) a first primer complimentary to a first strand of the double-stranded nucleic acid, said first primer having a first terminus complimentary to a first ligation site sequence of the first adaptor sequence, and (iv) a second primer complimentary to a second strand of the double-stranded nucleic acid, said second primer having a second terminus complimentary to a second ligation site sequence of a second adaptor sequence, wherein the first terminus of the first primer and the second terminus of the second primer are not identical;
  - b. treating the amplification product with the exonuclease to form a digested amplicon having a first overhang sequence at a first termini and a second overhang sequence at a second termini, wherein the first and second termini of said digested amplicon terminate at the sites of incorporation of said modified dNTPs; and

- c. ligating the first overhang sequence of the digested amplicon to the first ligation site sequence of the first adaptor sequence.
2. The process of claim 1 wherein the first adaptor sequence comprises a nucleotide sequence encoding at least one epitope tag.
  3. The process of claim 2 wherein the at least one epitope tag comprises *c-myc*, polyhistidine, polyarginine, glutathione-S-transferase (GST) tag, HA epitope, V5, Xpress™, and FLAG® epitope.
  4. The process of claim 3 wherein the at least one epitope tag comprises the FLAG® epitope.
  5. The process of claim 1 whereby at least 80% of the first overhang sequence of the digested amplicons are ligated to the first ligation site sequence of the first adaptor sequence.
  6. The process of claim 1 wherein the process further comprises ligating the second overhang sequence of the digested amplicon to the second ligation site sequence of the second adaptor sequence.
  7. The process of claim 6 wherein the first ligation site sequence and the second ligation site sequence are ends of a cloning vector.
  8. The process of claim 7 wherein at least 80% of the digested amplicons are inserted in only one direction in said vector.

9. The process of claim 1 wherein the first terminus of the first primer is a 3' terminus and the second terminus of the second primer is a 3' terminus.
10. The process of claim 1 wherein the first terminus of the first primer is a 5' terminus and the second terminus of the second primer is a 5' terminus.
11. The process of claim 10 wherein the exonuclease is a 3' to 5' exonuclease.
12. The process of claim 11 wherein the 3' to 5' exonuclease is exonuclease III.
13. The process of claim 1 wherein the modified dNTPs are alpha phosphate modified dNTPs.
14. The process of claim 13 wherein the alpha phosphate modified dNTPs are alpha phosphate thio-substituted dNTPs or alpha phosphate borano-substituted dNTPs.
15. The process of claim 14 wherein the alpha phosphate modified dNTPs are alpha phosphate modified purines or alpha phosphate modified pyrimidines.
16. The process of claim 15 wherein the alpha phosphate modified dNTPs are alpha phosphate modified dATP or alpha phosphate modified dGTP.
17. The process of claim 16 wherein the alpha phosphate modified dATP or alpha phosphate modified dGTP are alpha thiophosphorano dATP, alpha thiophosphorano dGTP, alpha

boranophosphorano dATP or alpha boranophosphorano dGTP.

18. The process of claim 17 wherein the alpha phosphate modified dATP or dGTP are alpha boranophosphorano dATP or alpha boranophosphorano dGTP.
19. The process of claim 1 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub>.
20. The process of claim 19 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is less than 20.
21. The process of claim 20 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is less than 9.
22. The process of claim 21 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is less than 1.
23. The process of claim 22 wherein modified dNTP<sub>1</sub> are alpha phosphate substituted dNTPs.
24. The process of claim 23 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is about 0.05 to 20.
25. The process of claim 24 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the

concentration of non-modified dNTP<sub>1</sub> is about 0.05 to 10.

26. The process of claim 25 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is about 0.05 to 4.
27. The process of claim 26 wherein modified dNTP<sub>1</sub> is alpha thiophosphorano dATP, alpha thiophosphorano dGTP, alpha boranophosphorano dATP or alpha boranophosphorano dGTP.
28. The process of claim 27 wherein modified dNTP<sub>1</sub> is alpha boranophosphorano dATP.
29. The process of claim 28 wherein the ratio of the concentration of alpha boranophosphorano dATP relative to the concentration of non-modified dATP is 0.05 to 10.
30. The process of claim 27 wherein modified dNTP<sub>1</sub> is alpha boranophosphorano dGTP.
31. The process of claim 30 wherein the ratio of the concentration of alpha boranophosphorano dGTP relative to the concentration of non-modified dGTP is 0.05 to 20.
32. The process of claim 1 wherein the dNTP mixture comprises modified dNTPs for two of the four nucleotide triphosphates.
33. The process of claim 32 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to

5 the concentration of non-modified  $\text{dNTP}_1$  to the concentration of non-modified  $\text{dNTP}_2$ , wherein  $\text{dNTP}_1$  and  $\text{dNTP}_2$  is dATP, dCTP, dGTP or dTTP, provided that  $\text{dNTP}_1$  and  $\text{dNTP}_2$  are not identical.

34. The process of claim 33 wherein the ratio of the concentration of modified  $\text{dNTP}_1$  to the concentration of modified  $\text{dNTP}_2$  relative to the concentration of non-modified  $\text{dNTP}_1$  to the concentration of non-modified  $\text{dNTP}_2$  is less than 51.

35. The process of claim 34 wherein the ratio of the concentration of modified  $\text{dNTP}_1$  to the concentration of modified  $\text{dNTP}_2$  relative to the concentration of non-modified  $\text{dNTP}_1$  to the concentration of non-modified  $\text{dNTP}_2$  is less than 27.

36. The process of claim 35 wherein the ratio of the concentration of modified  $\text{dNTP}_1$  to the concentration of modified  $\text{dNTP}_2$  relative to the concentration of non-modified  $\text{dNTP}_1$  to the concentration of non-modified  $\text{dNTP}_2$  is less than 13.

37. The process of claim 36 wherein modified  $\text{dNTP}_1$  and modified  $\text{dNTP}_2$  are alpha phosphate substituted dNTPs.

38. The process of claim 37 wherein the ratio of the concentration of modified  $\text{dNTP}_1$  to the concentration of modified  $\text{dNTP}_2$  relative to the concentration of non-modified  $\text{dNTP}_1$  to the concentration of non-modified  $\text{dNTP}_2$  is about 0.05 to 6.4.

39. The process of claim 38 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.1 to 3.2.
40. The process of claim 39 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.2 to 1.6.
41. The process of claim 40 wherein dNTP<sub>1</sub> is dGTP and dNTP<sub>2</sub> is dATP.
42. The process of claim 41 wherein modified dGTP is alpha thiophosphorano dGTP and modified dATP is alpha thiophosphorano dATP.
43. The process of claim 42 wherein the ratio of the concentration of alpha thiophosphorano dGTP to the concentration of alpha thiophosphorano dATP relative to the concentration of non-modified dGTP to the concentration of non-modified dATP is about 0.66.
44. The process of claim 41 wherein modified dGTP is alpha boranophosphorano dGTP and modified dATP is alpha boranophosphorano dATP.
45. The process of claim 44 wherein the ratio of the concentration of alpha boranophosphorano dGTP to the concentration of alpha boranophosphorano dATP relative to

the concentration of non-modified dGTP to the  
concentration of non-modified dATP is about 0.4.

46. A kit for directionally ligating a double-stranded nucleic acid to a first adaptor sequence, said kit comprising:
- a. a deoxynucleotidetriphosphate (dNTP) mixture, the dNTP mixture comprising modified dNTPs for at least one of the four nucleotide triphosphates comprising dATP, dGTP, dCTP, dTTP and analogs thereof which, when incorporated into a polynucleotide, impart resistance against enzymatic degradation by an exonuclease at the site of incorporation of the modified dNTPs; and
  - b. instructions for using said dNTP mixture to ligate said nucleic acid into the first adaptor sequence.
47. The kit of claim 46 wherein the kit further comprises the first adaptor sequence, wherein said first adaptor sequence comprises a nucleotide sequence encoding at least one epitope tag.
48. The process of claim 47 wherein the at least one epitope tag comprises *c-myc*, polyhistidine, polyarginine, glutathione-S-transferase (GST) tag, HA epitope, V5, Xpress™, and FLAG® epitope.
49. The process of claim 48 wherein the at least one epitope tag comprises the FLAG® epitope.
50. The kit of claim 46 wherein the kit further comprises instructions for using said dNTP mixture to ligate said nucleic acid into a second adaptor sequence.



51. The kit of claim 46 wherein said kit further comprises the exonuclease.
52. The kit of claim 51 wherein said exonuclease is a 3' to 5' exonuclease.
53. The kit of claim 52 wherein the exonuclease is exonuclease III.
54. The kit of claim 46 wherein the modified dNTPs are alpha phosphate modified dNTPs.
55. The kit of claim 54 wherein the alpha phosphate modified dNTPs are alpha phosphate thio-substituted dNTPs or alpha phosphate borano-substituted dNTPs.
56. The kit of claim 55 wherein the alpha phosphate modified nucleotides are alpha phosphate modified purines or alpha phosphate modified pyrimidines.
57. The kit of claim 56 wherein the alpha phosphate modified nucleotides are alpha phosphate modified dATP or dGTP.
58. The kit of claim 57 wherein the alpha phosphate modified nucleotides are alpha thiophosphorano dATP, alpha thiophosphorano dGTP, alpha boranophosphorano dATP or alpha boranophosphorano dGTP.
59. The kit of claim 58 wherein the alpha phosphate modified nucleotides are alpha thiophosphorano dATP or alpha thiophosphorano dGTP.

60. The kit of claim 58 wherein the alpha phosphate modified dATP and dGTP are alpha boranophosphorano dATP and dGTP.
61. The kit of claim 46 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub>.
62. The kit of claim 61 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is less than 20.
63. The kit of claim 62 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is less than 9.
64. The kit of claim 63 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is less than 1.
65. The kit of claim 46 wherein modified dNTP<sub>1</sub> are alpha phosphate substituted dNTPs.
66. The kit of claim 65 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is about 0.05 to 20.
67. The kit of claim 66 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is about 0.05 to 10.

68. The kit of claim 67 wherein the ratio of the concentration of modified dNTP<sub>1</sub> relative to the concentration of non-modified dNTP<sub>1</sub> is about 0.05 to 4.
69. The kit of claim 68 wherein modified dNTP<sub>1</sub> is alpha thiophosphorano dATP, alpha thiophosphorano dGTP, alpha boranophosphorano dATP or alpha boranophosphorano dGTP.
70. The kit of claim 69 wherein modified dNTP<sub>1</sub> is alpha boranophosphorano dATP.
71. The kit of claim 70 wherein the ratio of the concentration of alpha boranophosphorano dATP relative to the concentration of non-modified dATP is 0.05 to 10.
72. The kit of claim 69 wherein modified dNTP<sub>1</sub> is alpha boranophosphorano dGTP.
73. The kit of claim 72 wherein the ratio of the concentration of alpha boranophosphorano dGTP relative to the concentration of non-modified dGTP is 0.05 to 20.
74. The kit of claim 46 wherein the dNTP mixture comprises modified dNTPs for two of the four nucleotide triphosphates.
75. The kit of claim 74 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub>, wherein dNTP<sub>1</sub> and

dNTP<sub>2</sub> is dATP, dCTP, dGTP or dTTP provided that dNTP<sub>1</sub> and dNTP<sub>2</sub> are not identical.

76. The kit of claim 75 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub>.

77. The kit of claim 76 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 51.

78. The kit of claim 77 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 27.

79. The kit of claim 78 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 13.

80. The kit of claim 79 wherein modified dNTP<sub>1</sub> and modified dNTP<sub>2</sub> are alpha phosphate substituted dNTPs.

81. The kit of claim 80 wherein the ratio of the

concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.05 to 6.4.

82. The kit of claim 81 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.1 to 3.2.

83. The kit of claim 82 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.2 to 1.6.

84. The kit of claim 83 wherein dNTP<sub>1</sub> is dGTP and dNTP<sub>2</sub> is dATP.

85. The kit of claim 84 wherein modified dGTP is alpha thiophosphorano dGTP and modified dATP is alpha thiophosphorano dATP.

86. The kit of claim 85 wherein the ratio of the concentration of alpha thiophosphorano dGTP to the concentration of alpha thiophosphorano dATP relative to the concentration of non-modified dGTP to the concentration of non-modified dATP is about 0.66.

87. The kit of claim 84 wherein modified dGTP is alpha boranophosphorano dGTP and modified dATP is alpha

boranophosphorano dATP.

88. The kit of claim 87 wherein the ratio of the concentration of alpha boranophosphorano dGTP to the concentration of alpha boranophosphorano dATP relative to the concentration of non-modified dGTP to the concentration of non-modified dATP is about 0.4.

89. A process for preparing amplicons from a double-stranded nucleic acid, the process comprising:

- a. annealing a first primer to a first strand of the nucleic acid, the first primer complimentary to a portion of said first strand of the nucleic acid and having a first terminus complimentary to a first ligation site sequence, and a second primer to a second strand of the nucleic acid, said second primer complimentary to a portion of the second strand of said nucleic acid and having a second terminus complimentary to a second ligation site sequence; and
- b. forming amplicons by extending each primer using a polymerase and a dNTP mixture comprising modified dNTPs for at least two of the four nucleotide tri-phosphates comprising dATP, dGTP, dCTP, TTP and analogs thereof which, when incorporated into a polynucleotide, impart resistance against enzymatic degradation by an exonuclease at the site of incorporation of the modified dNTPs.

90. The process of claim 89 wherein the first and second ligation site sequences are not identical to each other.

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91. The process of claim 89 wherein the first and second ligation site sequences are identical to each other.
  92. The process of claim 89 further comprising treating the amplicons with said exonuclease to form digested amplicons, wherein both termini of said digested amplicons terminate at sites of incorporation of said modified dNTPs.
  93. The process of claim 89 wherein the first terminus of the first primer is a 3' terminus and the second terminus of the second primer is a 3' terminus.
  94. The process of claim 89 wherein the first terminus of the first primer is a 5' terminus and the second terminus of the second primer is a 5' terminus.
  95. The process of claim 94 wherein the exonuclease is a 3' to 5' exonuclease.
  96. The process of claim 95 wherein the 3' to 5' exonuclease is exonuclease III.
  97. The process of claim 89 wherein the modified dNTPs are alpha phosphate modified dNTPs.
  98. The process of claim 97 wherein the alpha phosphate modified dNTPs are alpha phosphate thio-substituted dNTPs or alpha phosphate borano-substituted dNTPs.
  99. The process of claim 98 wherein the alpha phosphate modified nucleotides are alpha phosphate modified purines or alpha phosphate modified pyrimidines.

100. The process of claim 99 wherein the alpha phosphate modified nucleotides are alpha phosphate modified dATP and dGTP.
101. The process of claim 100 wherein the alpha phosphate modified dATP and dGTP are alpha thiophosphorano dATP and dGTP or alpha boranophosphorano dATP and dGTP.
102. The process of claim 101 wherein the alpha phosphate modified dATP and dGTP are alpha thiophosphorano dATP and dGTP.
103. The process of claim 101 wherein the alpha phosphate modified dATP and dGTP are alpha boranophosphorano dATP and dGTP.
104. The process of claim 89 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub>, wherein dNTP<sub>1</sub> and dNTP<sub>2</sub> is dATP, dCTP, dGTP or dTTP, provided that dNTP<sub>1</sub> and dNTP<sub>2</sub> are not identical.
105. The process of claim 104 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub>.
106. The process of claim 105 wherein the ratio of the



concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 51.

107. The process of claim 106 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 27.

108. The process of claim 107 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 13.

109. The process of claim 108 wherein modified dNTP<sub>1</sub> and modified dNTP<sub>2</sub> are alpha phosphate substituted dNTPs.

110. The process of claim 109 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.05 to 6.4.

111. The process of claim 110 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.1 to 3.2.

112. The process of claim 111 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.2 to 1.6.

113. The process of claim 112 wherein dNTP<sub>1</sub> is dGTP and dNTP<sub>2</sub> is dATP.

114. The process of claim 113 wherein modified dGTP is alpha thiophosphorano dGTP and modified dATP is alpha thiophosphorano dATP.

115. The process of claim 114 wherein the ratio of the concentration of alpha thiophosphorano dGTP to the concentration of alpha thiophosphorano dATP relative to the concentration of non-modified dGTP to the concentration of non-modified dATP is about 0.66.

116. The process of claim 113 wherein modified dGTP is alpha boranophosphorano dGTP and modified dATP is alpha boranophosphorano dATP.

117. The process of claim 116 wherein the ratio of the concentration of alpha boranophosphorano dGTP to the concentration of alpha boranophosphorano dATP relative to the concentration of non-modified dGTP to the concentration of non-modified dATP is about 0.4.

118. The process of claim 89 wherein the first ligation site sequence is an Acc65I, AflIII, AgeI, AcaI, ApoI, AvrII, BamHI, BglIII, BsiWI, EagI, EcoRI, HindIII, NcoI, NgoMIV,

5 NheI, NotI, SalI, XbaI, XhoI or XmaI recognition sequence, and the second ligation site sequence is an Acc65I, AflII, AgeI, AcaI, ApoI, AvrII, BamHI, BglII, BsiWI, EagI, EcoRI, HindIII, NcoI, NgoMIV, NheI, NotI, SalI, XbaI, XhoI or XmaI recognition sequence.

119. The process of claim 118 wherein the first ligation site sequence is an EcoRI recognition sequence.
120. The process of claim 118 wherein the first ligation site sequence is an XbaI recognition sequence, and the second ligation site sequence is a BamHI recognition sequence.
121. An amplicon comprising a double-stranded, amplified nucleic acid fragment, the nucleic acid fragment comprising at least two modified dNTPs incorporated into the amplicon, wherein the nucleic acid fragment is resistant to enzymatic degradation by an exonuclease at the site of incorporation of the at least two modified dNTPs, a first terminus complimentary to a first ligation site sequence, and a second terminus complimentary to a second ligation site sequence.
122. The amplicon of claim 121 wherein the first and second ligation site sequences are not identical to each other.
123. A vector comprising the amplicon of claim 121.
124. The amplicon of claim 122 further comprising a first adaptor sequence comprising a nucleotide sequence encoding for at least one epitope tag.
125. The amplicon of claim 124 wherein the at least one epitope tag comprises *c-myc*, polyhistidine, polyarginine,

glutathione-S-transferase (GST) tag, HA epitope, V5, Xpress™, and FLAG® epitope.

126. The process of claim 125 wherein the at least one epitope tag comprises the FLAG® epitope.
127. A vector comprising the amplicon of claim 126.
128. The amplicon of claim 121 wherein the first and second termini of the amplicon are 3' termini.
129. The amplicon of claim 121 wherein the amplicon has blunt-ended termini.
130. The amplicon of claim 121 wherein the amplicon further comprises a first single stranded overhang sequence at one 5' terminus end which is complimentary to the first ligation site sequence, a second single stranded overhang sequence at a second 5' terminus end which is complimentary to the second ligation site sequence.
131. The amplicon of claim 130 wherein the first and second ligation site sequences are not identical to each other.
132. The amplicon of claim 130, the amplicon further comprising 3' termini which terminate at sites of incorporation of the modified dNTPs.
133. The amplicon of claim 132 wherein the first ligation site sequence is an Acc65I, AflIII, AgeI, AcaI, ApoI, AvrII, BamHI, BglIII, BsiWI, EagI, EcoRI, HindIII, NcoI, NgoMIV, NheI, NotI, SalI, XbaI, XhoI or XmaI recognition sequence, and the second ligation site sequence is an Acc65I, AflIII, AgeI, AcaI, ApoI, AvrII, BamHI, BglIII,

BsiWI, EagI, EcoRI, HindIII, NcoI, NgoMIV, NheI, NotI, SalI, XbaI, XhoI or XmaI recognition sequence.

134. The amplicon of claim 132 wherein the first ligation site sequence is an EcoRI recognition sequence.
135. The amplicon of claim 132 wherein the first ligation site sequence is an XbaI recognition sequence, and the second ligation site sequence is a BamHI recognition sequence.
136. A deoxynucleotidetriphosphate (dNTP) mixture comprising modified dNTPs for at least two of the four nucleotide triphosphates, wherein said modified dNTPs comprise alpha phosphate modified purines or alpha phosphate modified pyrimidines.
137. The dNTP mixture of claim 136 wherein the modified dNTPs comprise alpha phosphate thio-substituted dNTPs or alpha phosphate borano-substituted dNTPs.
138. The dNTP mixture of claim 137 wherein the modified dNTPs are alpha thiophosphorano dATP and dGTP or alpha boranophosphorano dATP and dGTP.
139. The dNTP mixture of claim 138 wherein the modified dNTPs are alpha thiophosphorano dATP and dGTP.
140. The dNTP mixture of claim 138 wherein the modified dNTPs are alpha boranophosphorano dATP and dGTP.
141. The dNTP mixture of claim 136 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to

5 the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub>, wherein dNTP<sub>1</sub> and dNTP<sub>2</sub> is dATP, dCTP, dGTP or dTTP, provided that dNTP<sub>1</sub> and dNTP<sub>2</sub> are not identical.

142. The dNTP mixture of claim 141 wherein the amount of the four nucleotide triphosphates in the dNTP mixture is determined by the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub>.

143. The dNTP mixture of claim 142 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 51.

144. The dNTP mixture of claim 143 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 27.

145. The dNTP mixture of claim 144 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is less than 13.

146. The dNTP mixture of claim 145 wherein modified dNTP<sub>1</sub> and modified dNTP<sub>2</sub> are alpha phosphate substituted dNTPs.

147. The dNTP mixture of claim 146 wherein the ratio of the

concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.05 to 6.4.

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148. The dNTP mixture of claim 147 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.1 to 3.2.

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149. The dNTP mixture of claim 148 wherein the ratio of the concentration of modified dNTP<sub>1</sub> to the concentration of modified dNTP<sub>2</sub> relative to the concentration of non-modified dNTP<sub>1</sub> to the concentration of non-modified dNTP<sub>2</sub> is about 0.2 to 1.6.

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150. The dNTP mixture of claim 149 wherein dNTP<sub>1</sub> is dGTP and dNTP<sub>2</sub> is dATP.

151. The dNTP mixture of claim 150 wherein modified dGTP is alpha thiophosphorano dGTP and modified dATP is alpha thiophosphorano dATP.

152. The dNTP mixture of claim 151 wherein the ratio of the concentration of alpha thiophosphorano dGTP to the concentration of alpha thiophosphorano dATP relative to the concentration of non-modified dGTP to the concentration of non-modified dATP is about 0.66.

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153. The dNTP mixture of claim 150 wherein modified dGTP is alpha boranophosphorano dGTP and modified dATP is alpha boranophosphorano dATP.

154. The dNTP mixture of claim 153 wherein the ratio of the  
concentration of alpha boranophosphorano dGTP to the  
concentration of alpha boranophosphorano dATP relative to  
the concentration of non-modified dGTP to the  
concentration of non-modified dATP is about 0.4.

155. A process for cloning a nucleic acid into a vector, the  
process comprising:

- a. forming an amplification product from the double-stranded nucleic acid using a mixture comprising  
(i) a polymerase, (ii) a deoxynucleotidetriphosphate (dNTP) mixture, the dNTP mixture comprising modified dNTPs for at least two of the four nucleotide triphosphates comprising dATP, dGTP, dCTP, dTTP and analogs thereof which, when incorporated into a polynucleotide, impart resistance against enzymatic degradation by an exonuclease at the site of incorporation of the modified dNTPs, (iii) a first primer complimentary to a first strand of the double-stranded nucleic acid, said first primer having a first terminus complimentary to a first ligation site sequence of the vector, and (iv) a second primer complimentary to a second strand of the double-stranded nucleic acid, said second primer having a second terminus complimentary to a second ligation site sequence of the vector;
- b. treating the amplification product with the exonuclease to form a digested amplicon, wherein both termini of said digested amplicon terminate at the sites of incorporation of said modified dNTPs; and
- c. ligating the digested amplicon to a first and second



ligation site sequence of the cloning vector.

156. The process of claim 155 wherein said process further comprises:
- d. transforming a host cell with said vector; and
  - e. identifying a clone of host cells that contains the digested amplicon in said vector.

157. The process of claim 155 wherein the first and second ligation site of the cloning vector are not identical.

158. The process of claim 157 wherein at least 80% of the digested amplicons are ligated in only one direction to the first and second ligation site sequences of said vector.